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Supplemental Material

IL-33 Drives Augmented Responses to Ozone in Obese Mice

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Supplemental Methods

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Figure S1. O₃-induced AHR is augmented in obese mice and reduced by anti-ST2. Changes in the coefficients of A) lung tissue damping (G) and B) elastance (H) and in C) Newtonian resistance (R_n) induced by inhaled aerosolized methacholine in lean wildtype (WT) and obese *db/db* female mice exposed to air or ozone (O₃) (2 ppm for 3 h) and studied 24 h after exposure. D) Changes in pulmonary resistance (R_L) in *db/db* and WT mice treated with isotype or anti-ST2 antibody prior to O₃ exposure. E) Methacholine-induced changes in G in *db/db* mice treated with either anti-ST2 or isotype antibody and exposed to air. Results are mean \pm SE of 4-8 mice/group.

Figure S2. Effect of anti-CD4 on BAL cytokines in obese-O₃ exposed mice. Changes in total lung CD4⁺ cells (A), and in BAL IL-5 (B), IL-9 (C), KC (D), IL-6 (E), CCL4 (F), Airway hyperresponsiveness (G) and BAL neutrophils (H) in *db/db* mice treated with anti-CD4 or isotype antibody prior to O₃ exposure. Results are mean \pm SE of 4-6 mice/group studied over 4 experimental days.

Figure S3. Gating of ILC2. Lungs were disrupted, and single cell suspensions generated.

Unstimulated cells were stained with CD45, Thy1.2, a lineage cocktail, ST2, and CD127. In A-D are shown stains from one representative *db/db* mouse exposed to O₃. The fluorescence minus one (FMO) control stains for Lin, Thy1.2, ST2, and CD127 are also shown. For control staining, cells from three *db/db* O₃-exposed mice were combined. Total ILC2 (Figure 4A) were gated as SSC^{Low}FSC^{Low}CD45⁺Lin⁻Thy1.2⁺ST2⁺CD127⁺.

Figure S4. Gating for IL-13⁺ and IL-5⁺ ILC2. Lungs were disrupted, single cell suspensions generated, and cells stimulated with PMA and ionomycin as described in Supplemental Methods. Shown is a representative stain from one *db/db* O₃-exposed mouse. Cells were stained with antibodies to CD45, Thy1.2, a lineage (Lin) cocktail, IL-5 or IL-13, and ST2. IL-13⁺ and IL-5⁺ ILC2s were gated as SSC^{Low}FSC^{Low}CD45⁺Lin⁻Thy1.2⁺IL-13⁺ and SSC^{Low}FSC^{Low}CD45⁺Lin⁻Thy1.2⁺IL-5⁺ respectively. As confirmation of the cells ILC2 state, cells were also stained with ST2, shown in the upper right quadrant are those cells that are SSC^{Low}FSC^{Low}CD45⁺Lin⁻Thy1.2⁺IL-13⁺ST2^{High} or SSC^{Low}FSC^{Low}CD45⁺Lin⁻Thy1.2⁺IL-5⁺ST2^{High} which the majority were. The fluorescence minus one (FMO) control stains for IL-13 and IL-5 (which used the same isotype) are also shown. For control staining, from three *db/db* O₃-exposed mice were combined.

Figure S5. IL-13⁺ST2⁺ costaining on γδ T cells. Lungs were disrupted, single cell suspensions generated, and cells stimulated with PMA and ionomycin as described in Supplemental Methods. Cells were stained with antibodies to CD45, CD3, TCRδ, IL-13, and ST2. IL-13⁺ γδ T cells (Figure 4C,E) were gated as SSC^{low}CD45⁺CD3⁺TCRδ⁺IL-13⁺ and ST2⁺ γδ T cells were gated as SSC^{low}TCRδ⁺ST2⁺ (Figure 4C,D). To determine if the IL-13⁺ γδ T cells were also positive for ST2, cells were gated as SSC^{low}CD45⁺CD3⁺TCRδ⁺IL-13⁺ST2⁺. Shown is one representative stain from a *db/db* mouse exposed to O₃. For the fluorescence minus one (FMO) control stains, cells from three *db/db* O₃-exposed mice were combined.